review

The emerging role of group VI calcium-independent phospholipase A_2 in releasing docosahexaenoic acid from brain phospholipids

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Abstract **Brain phospholipids are highly enriched in docosahexaenoic acid (DHA; 22:6n-3). Recent advances indicate that 22:6n-3 is released from brain phospholipids via the action of phospholipase A_2 (PLA_2) in response to several stimuli, including neurotransmission, where it then acts as a secondary messenger. Furthermore, it is now known that released 22:6n-3 is a substrate for several oxygenation enzymes whose products are potent signaling molecules. One emerging candidate PLA_2 involved in the release of 22:6n-3 from brain phospholipids is the group VI calcium-independent phospholipase A_2 ($iPLA_2$). After a brief review of brain 22:6n-3 metabolism, cell culture and rodent studies facilitating the hypothesis that group VI $iPLA_2$ releases 22:6n-3 from brain phospholipids are discussed. The identification of PLA_2s involved in cleaving 22:6n-3 from brain phospholipids could lead to the development of novel therapeutics for brain disorders in which 22:6n-3 signaling is disordered.**—Green, J. T., S. K. Orr, and R. P. Bazinet. **The emerging role of group VI calcium-independent phospholipase A_2 in releasing docosahexaenoic acid from brain phospholipids. *J. Lipid Res.* 2008.** 49: **939–944.**

Supplementary key words signaling • cyclooxygenase • lipoxygenase • docosanoid • neuroprotectin • uptake • turnover • kinetics • neuroinflammation • arachidonic acid

BRAIN UPTAKE AND UTILIZATION OF DOCOSAHEXAENOIC ACID

The mammalian brain is particularly enriched with the PUFA docosahexaenoic acid (DHA; 22:6n-3) (1). Within the brain, 22:6n-3 helps maintain membrane fluidity (2), promotes cell survival (3–5), acts as a secondary messenger via coupling to neuroreceptors (6–8), and is converted, via oxygenation, to a variety of signaling molecules, some of which have potent anti-inflammatory properties (9, 10). Thus, it is not surprising that 22:6n-3 is important in neural development (11, 12) and has been implicated in a variety of neurological disorders, including stroke (13), Alzheimer's disease (14), and major depression (15).

The brain cannot synthesize n-3 PUFAs de novo; therefore, it must either take up preformed 22:6n-3 or desaturate and elongate one of its precursors. With regard to the latter, although brain cells and in particular astrocytes have the capacity to desaturate and elongate α-linolenic acid (18:3n-3) to 22:6n-3 (16), in vivo tracer studies suggest that brain desaturation/elongation is a relatively minor pathway (0.024 pmol/g brain/s) (17) compared with brain uptake of preformed 22:6n-3 (13–15 pmol/g brain/s) (17–19). Furthermore, unlike the liver, the brain does not upregulate its ability to desaturate and elongate 18:3n-3 to 22:6n-3 when dietary n-3 PUFAs are limited (20–22).

Plasma unesterified 22:6n-3 rapidly disassociates from albumin, passes through the blood-brain barrier, and enters the brain (**Fig. 1**, step A) (23–25). Upon its entry, 22:6n-3 is activated by an acyl-CoA synthetase (26, 27). A small portion of the newly formed docosahexaenoyl-CoA is β-oxidized (28, 29), and the remainder is esterified via an acyl-CoA transferase to the *sn*-2 position of phospholipids. The 22:6n-3 that enters the brain and passes through the docosahexaenoyl-CoA pool is esterified into brain phospholipids at a rate of 13–15 pmol/g brain/s. This 22:6n-3 pool is predominantly esterified to ethanolamine (6–7 pmol/g brain/s) and choline glycerophospholipids (4–5 pmol/g brain/s) (28–31), and subsequent remodeling and de novo phospholipid synthesis likely explain its relative mass distribution within various phospholipid species (32). Phospholipid 22:6n-3 (*sn*-2 esterified) is then released by phospholipase A_2 (PLA_2) at a net rate of 102–131 pmol/g brain/s (29–31). A portion of the released 22:6n-3 is available for the synthesis of oxygen-

Abbreviations: 18:2n-6, linoleic acid; 20:4n-6, arachidonic acid; 22:6n-3, docosahexaenoic acid; $cPLA_2$, cytosolic phospholipase A_2; $iPLA_2$, calcium-independent phospholipase A_2; PLA_2, phospholipase A_2; $sPLA_2$, secretory phospholipase A_2.

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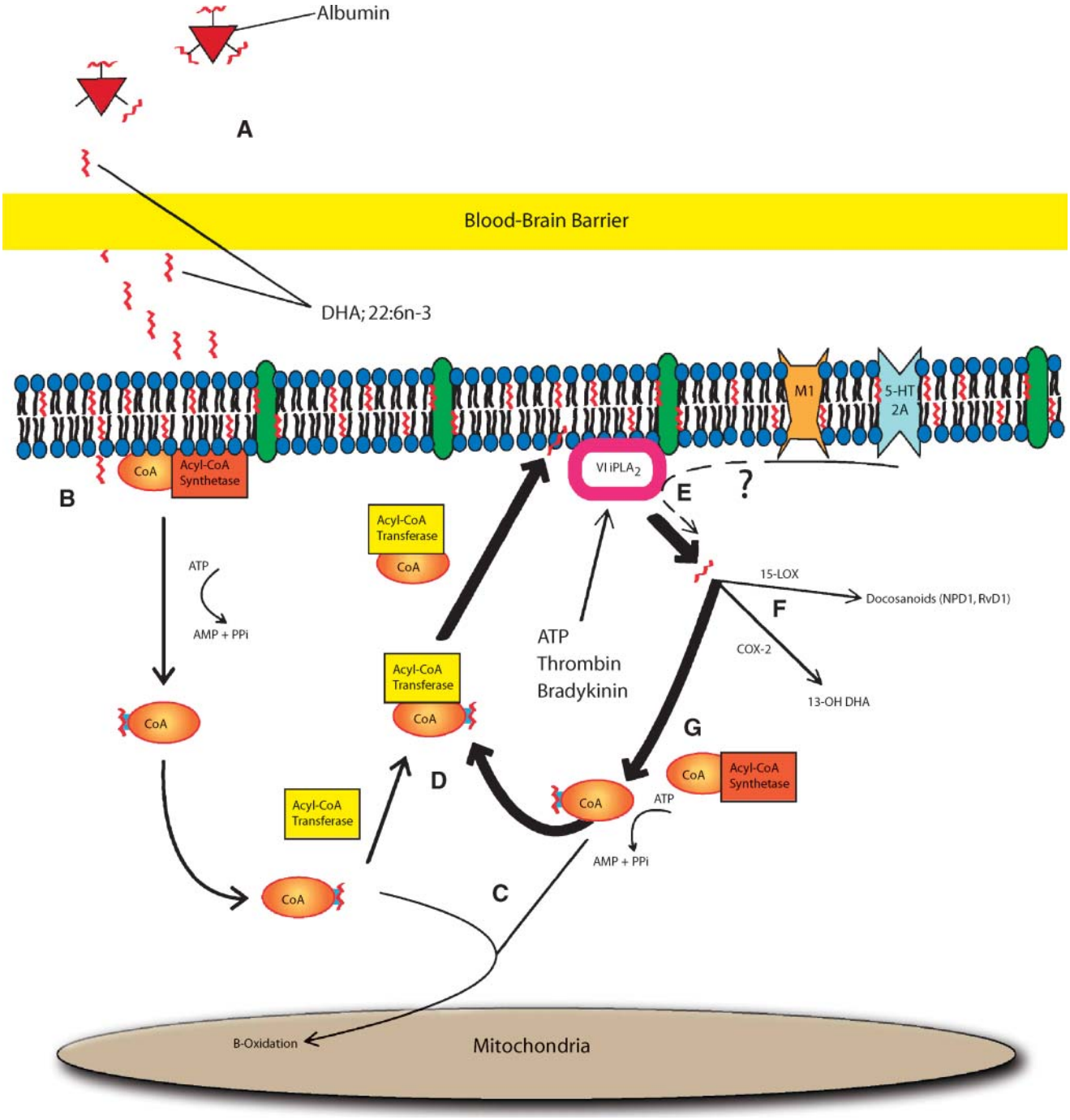

Fig. 1. Docosahexaenoic acid (DHA; 22:6n-3) rapidly disassociates from plasma albumin and passes through the blood-brain barrier (A) into the brain, where it is activated by an acyl-CoA synthetase (B). Upon its activation, a small proportion of 22:6n-3 can be β-oxidized (C), whereas the remainder is esterified to a 2-lysophospholipid via acyl-CoA transferase (D). In this model, group VI calcium-independent phospholipase A_2 ($iPLA_2$) releases 22:6n-3 from neural phospholipids (creating the 2-lysophospholipid) (E), where a portion can be converted to oxygenated derivatives (F) or activated by acyl-CoA synthetase (G). ATP, thrombin, and bradykinin are known activators of $iPLA_2$. Although cholinergic (M1) and serotonergic (5-HT2A) receptor activation releases 22:6n-3 from neural phospholipids, the PLA_2 isoform involved is not known. Plasma unesterified 22:6n-3 enters the brain and is esterified to a 2-lysophospholipid at a rate of 13–15 pmol/g brain/s. However, the net rate of 22:6n-3 esterification to brain phospholipids also includes recycled 22:6n-3, leading to a net rate of 22:6n-3 entry into phospholipids from the docosahexaenoyl-CoA pool (D) of 102–131 pmol/g brain/s, which at steady state approximates the rate of 22:6n-3 release from brain phospholipids (E). NPD1, neuroprotectin D1; RvD1, resolvin D1.

ated derivatives via cyclooxygenase-2 (33) or a putative 15-lipoxygenase (34), whereas the remainder is activated by an acyl-CoA synthetase in which, again, a small portion of it is available for β-oxidation and the remainder is reesterified into the *sn*-2 position of brain phospholipids. Kinetic studies estimate that under basal conditions, ~90% of the 22:6n-3 that is released via PLA_2 is reesterified into the *sn*-2 position of brain phospholipids (29–31), and the

10% that is lost is replaced by uptake from the plasma unesterified 22:6n-3 pool (35).

BRAIN PLA_2

To date, at least 22 genes that encode PLA_2 proteins have been identified in mammals. PLA_2s cleave fatty acids from the *sn*-2 position of glycerophospholipids, resulting in an unesterified "free fatty acid" and a 2-lysophospholipid. On a broad level, the mammalian PLA_2 isoforms differ with respect to their tissue and cellular distribution, substrate specificities, and calcium requirements, and interested readers should consult other reviews for more details (36–41), including PLA_2 nomenclature/classification (41, 42). The isoforms identified in the brain to date include groups IVA, IVB, and IVC calcium-dependent cytosolic phospholipase A_2 ($cPLA_2$), groups IIA, IIC, IIE, V, and X calcium-dependent secretory phospholipase A_2 ($sPLA_2$), and groups VIA and VIB calcium-independent phospholipase A_2 ($iPLA_2$) (43–49). It is worth noting that the mouse strains C57BL/6, 129/Sv, and B10.RIII have a naturally occurring missense mutation in the gene encoding for the group IIA $sPLA_2$ (45, 50–52). As new proteins with PLA_2-like activity are identified (53, 54) and specific antibodies and reagents are developed, it is possible that more isoforms will be detected within the brain. Another, yet to be cloned, PLA_2 whose activity has been detected in the brain is the plasmalogen-selective PLA_2 (55). This enzyme is capable of cleaving fatty acids from plasmanylethanolamine, and because in the brain this glycerophospholipid is rich in 22:6n-3, it is possible that this enzyme may also play a role in 22:6n-3 release from brain phospholipids (39, 56).

GROUP VI $iPLA_2$: OVERVIEW

There are two distinct members of the $iPLA_2$ family, group VIA $iPLA_2$ and group VIB $iPLA_2$. The 85–88 kDa group VIA $iPLA_2$ was first isolated from macrophages in 1994 (57) and independently cloned by two laboratories in 1997 (58, 59), whereas the group VIB $iPLA_2$ was cloned in 2000 (49). The group VIA $iPLA_2$ shares several conserved regions at the C terminus but has very little homology at the N terminus with the group VIB $iPLA_2$. The group VI $iPLA_2$s do not require calcium for their activity but do use ATP for stabilization; they are stimulated by thrombin and bradykinin (40, 60) but inhibited by bromoenolactone (61). However, very little is known about the molecular/genetic regulation of group VI $iPLA_2$, especially within the brain (62). Initial studies suggested a homeostatic role for group VI $iPLA_2$ in regulating membrane turnover (for review, see Ref. 63). However, several studies have now shown that group VI $iPLA_2$ plays an important role in signal transduction (for review, see Refs. 40, 64).

In the brain, the basal expression and activity of group VI $iPLA_2$ is higher than that of other PLA_2s (43, 65–67) and its protein expression decreases during aging (68). Whereas brain $cPLA_2$ and $sPLA_2$ are commonly thought to be selective for arachidonic acid (20:4n-6) release (64, 69–73), the specific activity of group VI $iPLA_2$ using 1-palmitoyl 2-*R*-phosphatidylcholine when linoleic acid (18:2n-6), palmitic acid, oleic acid, or 20:4n-6 were esterified in the *sn*-2 position (*R*) was 10.0, 4.3, 3.0, and 2.0 μmol/min/mg protein, respectively (65). However, it is important to consider cellular localization and substrate availability when trying to determine in vivo selectivity. The net rate of release of 18:2n-6 from brain phospholipids (19 pmol/g brain/s) was at least five times lower than that of 22:6n-3 (102–131 pmol/g brain/s), and when preformed 22:6n-3 is present in chow, the concentration of phospholipid esterified 18:2n-6 (987 nmol/g brain) was almost 15 times lower than that of 22:6n-3 (13,844 nmol/g brain) (18). Although these latter observations do not suggest that group VI $iPLA_2$ is not involved in cleaving 18:2n-6 from brain phospholipids, they do raise the question of why so much group VI $iPLA_2$ is present in the brain when so little 18:2n-6 is present.

GROUP VI $iPLA_2$ AND 22:6n-3 RELEASE FROM BRAIN PHOSPHOLIPIDS

Evidence from cell culture studies

The first suggestion that brain group VI $iPLA_2$ may be selective for 22:6n-3 release came from Strokin, Sergeeva, and Reiser (60) when they observed that bromoenolactone inhibited 22:6n-3 but not 20:4n-6 release from phospholipids of astrocytes stimulated with ATP. This study was followed up by showing that bromoenolactone also inhibited oxygen/glucose deprivation-induced 22:6n-3 release from hippocampal phospholipids (74). In 2007, Strokin, Sergeeva, and Reiser (48) repeated their 2003 finding in which ATP-stimulated 22:6n-3 release from astrocyte phospholipids was inhibited with bromoenolactone and reproduced this effect with small interfering RNA silencing of group VIB $iPLA_2$. This latter study addressed the issue of bromoenolactone inhibition selectivity and was followed up by further demonstrating that bromoenolactone inhibition of 22:6n-3 release from astrocyte phospholipids was absent upon silencing group VIB $iPLA_2$.

Evidence from in vivo studies

DeMar et al. (75) tested the half-life of [4,5-^{3}H]22:6n-3 upon its intracerebroventricular administration to rats that had consumed a diet either adequate or deprived of n-3 PUFAs for 15 weeks postweaning. The half-life of [4,5-^{3}H]22:6n-3 in brain phospholipids of rats consuming the n-3 PUFA-adequate diet was 33 days, whereas it was increased to 90 days in the deprived rats. This conservation of [4,5-^{3}H]22:6n-3 suggested that enzymes involved in the catabolism of 22:6n-3 must be downregulated in the brains of n-3 PUFA-deprived rats, and in a subsequent experiment candidate brain PLA_2 isoforms (IIA $sPLA_2$, IVA $cPLA_2$, and VIA $iPLA_2$) were examined (47). Whereas the activity, protein, and mRNA of group IVA $cPLA_2$ and group IIA $sPLA_2$ were upregulated in the brains of rats

consuming the n-3 PUFA-deprived diet, only the group VIA iPLA$_2$ isoform was downregulated, making it a candidate mechanism by which the brain half-life of [4,5-^{3}H]22:6n-3 was prolonged (47, 75). The activity, protein, and mRNA of group VIA iPLA$_2$ were downregulated; however, the mechanism by which group VIA iPLA$_2$ mRNA was downregulated was not explored, and whether or not there was a downregulation of group VIB iPLA$_2$ was not tested.

CONSEQUENCES OF 22:6n-3 RELEASE FROM BRAIN PHOSPHOLIPIDS

Although many important functions have been attributed to 22:6n-3 within the brain (2, 76–78), few studies have attempted to test whether these functions are related to released 22:6n-3. Rats deprived of dietary n-3 PUFAs to reduce brain concentrations have behavioral deficits (79, 80), altered neurotransmission (81, 82), decreased iPLA$_2$ activity and expression (47), and decreased 22:6n-3 release from brain phospholipids (75, 83). A portion of released 22:6n-3 can be converted to neuroprotectin D1 or resolvin D1 (Fig. 1, step F), two signaling molecules involved in brain cell survival and the resolution of inflammation (10, 13, 84). Docosahexaenoyl-CoA is a hepatic nuclear factor-4α ligand (85), whereas unesterified 22:6n-3 is a peroxisome proliferator-activated receptor ligand (86). Unesterified 22:6n-3 inhibits protein kinase C activity (87) and has been implicated in the regulation of brain nuclear factor-κB (13), p38 mitogen-activated protein kinase (5), Bcl-2 (84), and Akt (88) signaling pathways. Furthermore, the activation of 22:6n-3 by acyl-CoA synthetase (102–131 pmol/g brain/s) requires the use of two high-energy phosphates from one ATP (22:6n-3 + CoA + ATP → 22:6n-3-CoA + AMP + PP_i; Fig. 1, step G), consuming ~0.1% of the rodent brain's 208 nmol/g brain/s ATP (89–91). Future studies are needed to determine the consequences of this energetically expensive 22:6n-3 release and reesterification.

SUMMARY AND CONCLUSIONS

Recent studies have identified 22:6n-3 as an important secondary messenger within the brain. The PLA$_2$ isoform involved in 22:6n-3 release from neural phospholipids upon cholinergic (M1) (6, 8) and serotonergic (5-HT2A) (7, 92) stimulation is not known. Unesterified 22:6n-3 likely directly, as well as through its oxygenated derivatives, participates in signal transduction. Cell culture studies using small molecule inhibitors and small interfering RNAs as well as kinetic studies in the rodent brain suggest that group VI iPLA$_2$ is involved in cleaving 22:6n-3 from brain phospholipids (Fig. 1). Future studies are needed to identify the specific roles of group VIA and VIB iPLA$_2$ in 22:6n-3 release from brain phospholipids. Further approaches to answering these questions could include the study of *sn*-2 radiolabeled 22:6n-3 glycerophospholipids in group VI iPLA$_2$-specific assay systems, the generation and characterization of groups VIA and VIB iPLA$_2$ brain-specific knockouts, or the assessment of 22:6n-3 kinetics in other models with altered brain group VI iPLA$_2$ activity. To date, it is known that group VIA iPLA$_2$ decreases in the hippocampus in response to aging (68) and in the cortex in response to dietary n-3 PUFA deprivation (47). Whether or not decreased group VI iPLA$_2$ and its potential ability to regulate 22:6n-3 release from brain phospholipids are contributing factors in the susceptibility of the aged or the n-3 PUFA-deprived brain to disease remains to be tested.

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